IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| SERIAL NO. | : | 09/600,564 |
| APPLICANT | : | Florian KERN |
| FILED | : | 001602us/JH/ml |
| EXAMINER | : | K.S. Shahnan Shah |
| ART UNIT | : | 1645 |
| FOR | : | METHOD FOR IDENTIFYING T-CELL STIMULATING PROTEIN FRAGMENTS |

Hon. Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

September 15, 2004

DECLARATION PURSUANT TO 37 CFR § 1.132

Sir:

I, Florian Kern, of Berlin, Germany, am a research group leader at the Institute for Medical Immunology, at Charité – Universitätsmedizin Berlin, a well-known research institution. My vitae is shown in Exhibit I. My group works in the field of antigen specific T lymphocytes and has been involved in the development of assays based on short term stimulation of T lymphocytes with specific antigens, including short peptides derived from the amino acid sequence of pathogens including viruses. I am the principal author or senior-author of more than 15 Medline-indexed research articles in this field summarized in Exhibit II. I have carefully studied the article entitled *CD30 Induction and Cytokine Profiles in Hepatitis C Virus Core-Specific Peripheral Blood T Lymphocytes* that was published by R. Woitas et al. in the Journal of Immunology (1997) vol. 159: 1012-1018.

The above-named article describes methods used to identify peptides from Hepatitis-C Virus (HCV) core protein that stimulate T lymphocytes. When stimulated, T lymphocytes demonstrate increased cellular levels of marker proteins; e.g., the surface protein CD30, interferon-γ and one or more interleukins (e.g., IL-2, IL-4 and IL-10). The increase in

marker proteins was quantified by measuring the levels of CD30, interferon-γ and/or various interleukins in comparison to unstimulated T lymphocytes.

1. The method described in the article of Woitas et al. comprises contacting T lymphocytes with one or more short peptides corresponding to distinct regions of the HCV protein. Peptide-stimulated T lymphocytes were analyzed after immunolabeling of the T lymphocytes with fluorescent antibodies to form fluorescent immune complexes for each protein of interest. The basis of the assay is that the magnitude of the fluorescent signal measured by flow cytometry is proportional to the amount of fluorescent antibody-protein complex present, which in turn, reflects the amount of the protein being measured.

The immunolabeling and quantification of the fluorescent immune complexes was performed after a 40 hour incubation of T lymphocytes with the respective HCV peptides.

2. The peptide stimulation of the T lymphocytes leading to the induction of CD30, interferon-γ and/or interleukins was achieved by incubating the T lymphocytes with 10 µg/ml of synthetic HCV peptides, for 40 hours. This methodology is described in the reference on page 1013, column 2 (the respective paragraph is entitled "Cells and cell culture"), and the legend of figure 3.

No peptide-incubation times other than the 40 hour period, were examined.

3. Twelve hours prior to the end of the 40 hour peptide-incubation period, the drug monensin was added to the culture medium. The purpose of monensin was merely to decrease the rate of cytokine secretion from the cell, thereby increasing the intracellular concentration of the induced cytokine. The increased intracellular concentration of the cytokines enhances the sensitivity of the fluorescent signal obtained from cytokine-antibody immunecomplexes.

4. At the end of the 40 hour incubation period with HCV peptides, the T lymphocytes were prepared for flow cytometry by forming the fluorescent immune complexes after fixing the cells in paraformaldehyde and permeabilizing the cells with saponin. It is well known among investigators in the art that fixation kills the cells. Therefore, the fixed and

permeable cells can only be used for analysis of the fluorescent signal, and cannot be stimulated to any further extent by incubation with the antibodies used to label the cells.

In sum, the immunolabeling of the peptide-contacted T lymphocytes for detecting interferon-γ and/or various interleukins was performed in dead cells with openings in their cell membranes due to saponin treatment.

5. The immunolabeling method is provided in detail on page 1013, col. 2 (the respective paragraph is entitled "Three-color flow cytometry for intracytoplasmic staining of cytokines"). It is noteworthy that the fixed and permeabilized cells were incubated with each of the relevant antibodies for no more than 30 minutes. The length of this time period is clearly much shorter than the 40 hour incubation with the HCV peptides.

Further, it should be emphasized that the 30 minute incubation of antibodies with the fixed permeabilized T lymphocytes for flow cytometry is completely unrelated to the 40 hour HCV peptide incubation to stimulate the cells.

In addition, the incubation of T lymphocytes with the HCV peptides requires the cells to be alive. Thus, immunolabeling of dead cells with antibodies cannot have any effect on the level of peptide-induced stimulation.

6. The methods described in the article of Woitas et al. discussed above are distinct from those described in our patent application 09/600,564. For example, in the method used in the article of Woitas et al., the peptide-contacted cells are incubated for 40 hours. This period is sufficiently long to result in stimulation-induced apoptosis and cell death. This potential loss of healthy induced cells is likely to impair the accurate measurement of a given peptide's stimulatory properties in terms of the number of induced T lymphocytes.

Further, if the induced apoptosis were peptide-specific, a potential stimulatory peptide may provide a false negative result due to the selective depletion of the peptide's corresponding T lymphocyte subpopulation.

7. We have found that by using a six hour incubation with HCMV-peptides, T lymphocyte stimulation may be assessed under conditions where the experimental

results will not be significantly effected by the problems referred to in paragraph 8. In contrast, the method employed in the article of Woitas et al. does not provide these advantages. Thus, investigators in this field of research would not consider our method to be identical to the method provided in the article Woitas et al. .

8. Upon reviewing the patent examiner's reasons for rejecting the above-referenced application, it was apparent that the examiner incorrectly concluded that the methods used in the article of Woitas et al. encompassed our method. This conclusion is incorrect.

Apparently, the examiner believes that incubating the dead fixed cells with various antibodies for 30 minutes is the equivalent of a 30 minute incubation of live T lymphocytes with HCV peptides. Therefore, the examiner concluded that the stimulation of T lymphocytes with only 30 minute incubation periods with HCV peptides was disclosed by Woitas et al. This is completely incorrect. Antibodies are not the equivalents of HCV peptides; and dead fixed T lymphocytes cannot be stimulated. Therefore, examiner's assertion that the method disclosed by Woitas et al. used very short peptide incubations for T cell stimulation, and thus, encompasses our method, is incorrect.

There is no method or procedure disclosed in the article of Woitas et al. that is the equivalent of our method as described in the above-referenced patent application.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Sept. 15, 2004
Date

Florian Kern

Enclosures
Exhibit I and II